Sterility Check SOP

Flow Cytometry Core Facility

Ver. # 1.0
2024-11-08

Workflow Overview

Collect sheath fluids weekly directly from sorter stream and incubation for 48h to do the sterility check.

Prepare reagents ▶ Set up controls ▶ Collect samples ▶ Incubation ▶ Record the results ▶ Re-check if needed

Purpose

Instructions on how to carry out sterility check on sorters to check yeast and bacterial contamination in the system.

Supplies needed

- BD Difco YPD broth, Fisher Scientific, Cat (Prepare yeast nutrient media)
- LB broth, Fisher Scientific, Cat # BP 1426-500 (Prepare bacterial nutrient media)
- Tissue culture hood
- Pipette Controller
- Sterile Polystyrene Disposable Serological Pipets
- Falcon™ Round-Bottom Polypropylene 14ml sterile tube with cap, Fisher Scientific, Cat # 14-959-11B
- Shaking incubator
- Lab analytical scale

1. Prepare reagents

1. Weigh and add 50 g YPD broth powder to 1000 mL distilled water in an autoclave glass bottle. Swirl to mix thoroughly for at least 15s.
2. Add 20 capsules LB broth powder to 1000 mL distilled water in an autoclave glass bottle. Swirl to mix thoroughly for at least 15s.

3. Replace the caps to the two bottles and put new autoclave tape on the top of the caps. Leave the caps a little bit loose for pressure equalization when autoclave.
4. Put the two bottles in a basin filled with some water.
5. Autoclave the two reagents on the *Liquid Cycle Program* at 121C for 15 mins.
6. Take the two bottles out of autoclave when the cycle completes and leave them inside the tissue culture hood to cool down to room temperature. Tighten the caps when they are cool. Now both reagents are ready for use.

2. Set up control tubes

1. Turn on the power of the tissue culture hood and spray the surface with 70% Ethanol and clean it. Use pipette controller and sterile polystyrene disposable serological pipets to aliquot the reagents into 14ml sterile round-bottom polypropylene and label them, 3 mL per tube.

	YPD	LB
Positive Controls	2 tubes	2 tubes
Negative Controls	2 tubes	2 tubes

2. For positive controls, swabs from the lab floor or from the bottom of your shoes are good positive controls both for YPD and LB. Open and cap and dip the swabs deeply into the media of the control tubes for a couple of seconds and then replace the cap back.
3. For negative controls, leave them with the closed caps.

3. Collect samples

1. Clean the instrument as usual, 2 minutes with FACS Clean, 2 minutes with FACS Rinse and 2 minutes with distilled H2O.
2. Aliquot and label YPD and LB testing tubes inside tissue culture hood same as controls. Prepare two YPD tubes and two LB tubes for each instrument.
3. Open the door of the Biosafety cabinet and silence the *Alarm*. Open the door of the sorting chamber.

   Note: Please remember to increase AMS to 100% for 1 minute and then back to 20% to get rid of the residual aerosols before opening the door.
4. Open the 14 mL sterile tube cap and collect about 1mL sheath directly from the central stream and replace and tighten the cap afterwards.
5. Collect samples from all instruments.

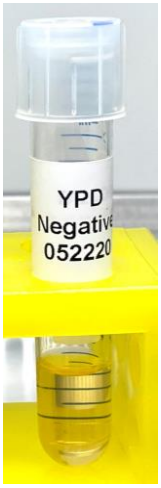
4. Incubation

1. Collect all sample tubes and control tubes in a rack.
2. Incubate them at 37°C for 48 hours with shaking.

5. Record the results

1. Remove the tubes out of incubator after 48 hours of incubation.

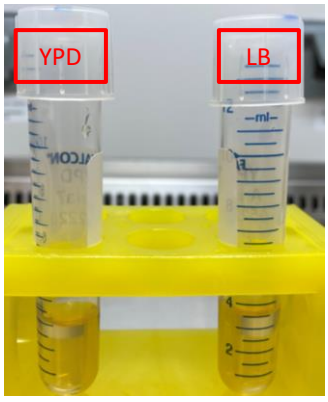
2. Check the sterility for the control tubes. Negative controls should be clear. Positive controls should be cloudy.



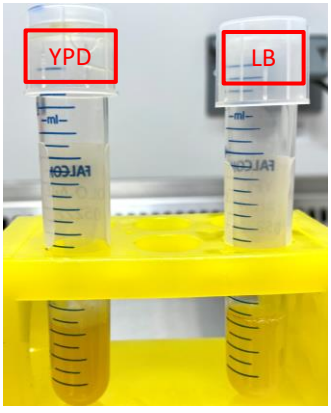
Negative control-clear

Positive control-cloudy

3. Check the sterility for sample tubes from all instruments.



Good sterility result-clear



Bad sterility result-cloudy

4. Record the testing data in the ***Sterility Checks File*** in the ***Sterility Checks Folder*** in ***FCCF*** server.

Week		Media			Yeast		
		1	2		1	2	
Aria 1							
Aria 3							
Aria 7							
Aria 5							
Aria 6							
Solo 2							
Solo 4							
Sony							
Sony RRL							

6. Re-check if needed

Re-check sterility may be needed if see any of the following results:

1. Contamination from one instrument or conflict results from the two tubes of the same instrument. Re-do sterility check for this instrument.
2. See positive results from all tubes including samples and negative controls. It indicates the possible contamination from the media. Prepare new media and re-do sterility check for all instruments.
3. See negative results from all tubes including samples and positive controls. It indicates the possible failure of the positive controls or expiration of the media. First re-check the positive and negative controls. If still get negative from both controls, make new media and re-do sterility check for all instruments.